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Phytochemical Analysis, α-glucosidase Inhibition Activity *in-vitro* and Enzyme Kinetics of Ethyl Acetate and Hexane Extracts of *Graptophylum pictum* (L.) Griff

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ABSTRACT

The species Graptophylum pictum (L.) Griff, also known as "daun ungu" in Indonesia, is a traditional herbaceous plant believed to have antidiabetic potential. The number of people in the world with diabetes has increased dramatically over the recent years. The treatment of type II diabetes is complicated by several factors inherent to the disease. Elevated postprandial hyperglycemia is one of the risk factors and the intestinal digestive enzyme α-glucosidase plays a vital role in carbohydrate metabolism. One of the antidiabetic therapeutic approaches which reduces the postprandial glucose level in blood is by the inhibition of α-glucosidase. In this study, phytochemical analysis, α-glucosidase inhibitory activity and enzyme kinetics of ethyl acetate- and hexane extracts of G. pictum were evaluated with the aim to analyze its antidiabetic potential. Phytochemical analysis revealed the presence of tannins, steroids, and alkaloids. Steroids were present in ethyl acetate extract but absent in hexane extract, while alkaloids were present in hexane extract but absent in ethyl acetate extract. The ethyl acetate and hexane extracts had 30.68 and 49.82% inhibitory effect on α-glucosidase activity respectively. The kinetics of glucosidase enzyme of ethyl acetate and hexane extracts were determined by Lineweaver Burk plots. These exhibited uncompetitive and noncompetitive inhibition to alpha-glucosidase activity respectively. From the enzyme assay, we infer that ethyl acetate and hexane extracts of G. pictum contain potential α-glucosidase inhibitors that have the potential to be exploited for use in the treatment of diabetes

Keywords: *Graptophyllum pictum (L.) Griff, α-glucosidase inhibitor activity, kinetics, diabetes*

1. INTRODUCTION

Diabetes is a chronic metabolic disorder in which homeostasis of the carbohydrate, protein and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level *i.e.* hyperglycemia (Nagmoti & Juvekar 2013). Danaei *et al*. (2011) estimated a total of 347 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar (WHO 2009) and it is predicted that diabetes will be the 7th leading cause of death in 2030 (WHO 2011). Glucose homeostasis is a key for the treatment of diabetes (Kaskoos 2013). The treatment of type II diabetes is complicated by several risk factors inherent to the disease (Kaskoos 2013). Elevated postprandial hyperglycemia is one of the risk factors (Gin & Rigalleau 2000). One important causes of postprandial hyperglycemia is the fast uptake of glucose in the intestine by the action of glucosidases, a class of enzymes that helps in the breakdown of complex carbohydrates (starch and oligosaccharides) into simple sugars such as maltose and glucose (Hua-Qiang *et al*. 2012; Gray 1995). Therefore one of the important therapeutic approaches to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes (α-glucosidase) (Nagmoti & Juvekar 2013). Several α-glucosidase inhibitors, such as acarbose (Schmidt *et al.* 1977), miglitol (Pogano *et al*. 1995), and valiolamine (Horii *et al*. 1987) have been isolated and used in the management of diabetes mellitus. However, these drugs are associated with gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients, which might be caused by excessive inhibition of pancreatic α-amylase resulting in fermentation of undigested carbohydrates in the colon by the colonic flora (Bhat *et al*. 2011; Suzuki *et al*. 2009). Thus searching for a new class of compounds is essential to address diabetes problems. The present work was undertaken to explore the antidiabetic potential of a plant *G. pictum* as a α-glucosidase enzyme inhibitor.

G. pictum, also known as "daun ungu" in Indonesia, is a medicinal plant from the family Acanthaceae distributed throughout Indonesia. The leaves have been used as traditional medicine for the treatment of constipation, rheumatism, menstruation, hemorrhoids, urinary infections, scabies, swelling, maturing boils, smoothing skin, wounds, dermatitis, hepatomegaly, ear disease, laxative, and chancre (PT Eisai Indonesia editor, 1995). Studies on extracts from leaves of this plant have revealed analgesic, anti-inflamatory (Ozaki *et al*. 1989) and hypoglycemic (Olangbede-Dada *et al*. 2011) activities. Research has shown that aqueous and 70% ethanol extracts of *G. pictum* have inhibitory activity against this α-glucosidase with a percentage inhibition value of 40.03 and 65.89% (Nurcholis *et al*. 2011). This shows that extracts of *G. pictum* can be developed as an antidiabetic. Based on these results, this study investigates the phytochemical, antidiabetic activity *in vitro* and enzyme kinetics of ethyl acetate and hexane extracts of *G. pictum*.

2. MATERIALS AND METHODS

Preparation of ethyl acetate and hexane extracts

Fresh leaves of *G. pictum* collected from The Conservation and Cultivation Unit

of Biopharmaca Research Center, Bogor Agricultural University, were washed with water, cut into small pieces and dried for 5 days under the sun (moisture: < 10%). They were then ground to powder form (size: 80 mesh). As much as 30 g of powdered leaves were macerated using 10 x 30 mL ethyl acetate and hexane in a tightly closed round bottomed flask at room temperature for a period of 24 h and filtered with Whatman filter paper (type 4). The whole process was repeated once and the filtrate was concentrated under reduced pressure on rotavapor (BUCHI, R-250, Switzerland) at 50°C. The resulted concentrated extracts were used for the following experiments.

Phytochemical analysis

The phytochemical composition of each extract was determined using standard phytochemical methods described by Harborne (1998).

α-Glucosidase inhibitory assay

The enzyme inhibitory activity toward α-glucosidase was evaluated according to the method previously reported by Shibano *et al*. (1997) with modification. The reaction mixture consisted of 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of 0.5 mM PNPG (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 µl of test sample (0.63%) /and or standard (acarbose) (1% w/v in DMSO) and 25 µl of α-glucosidase (Sigma-Aldrich, USA) solution (a stock solution of 1 mg/ml in 0.01 M phosphate buffer, pH 7.0, was diluted to 0.1 Unit/ml with the same buffer, pH 7.0, immediately prior to the assay). This reaction mixture was then incubated at 37°C for 30 min. The reaction was then terminated by the addition of 100 µl of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored based on the amount of p-nitrophenol released in the reaction mixture at 400 nm using a microplate reader. Inhibition percentage was calculated using the equation:

% Inhibition = [(C – S)/ C] x 100%

with S = the absorbance of sample (S1-S0; with S1 = absorbance of samples with enzyme addition and S0 = absorbance of sample without enzyme addition) and C = absorbance of control solution (DMSO), without sample (blank).

Kinetics Analysis of α-glucosidase inhibiton

To determine the inhibitory activity of the ethyl acetate- and hexane extracts of *G. pictum* against α-glucosidase, increasing concentrations of PNPG (4-nitrophenyl α-D-glucopyranoside) was used as substrate in the absence and presence of plant extracts at different concentrations. The parameters of α-glucosidase inhibiton was determined by Lineweaver-Burk plot analysis of the data (Lineweaver & Burk 1934), which were calculated from the results according to the Michaelis Menten kinetics.

3. RESULTS

Extract of *G. pictum* leaves

The yield of the extraction process of *G. pictum* leaves using ethyl acetate and hexane was 0.85 g (2.8% w/w) and 0.59 g (2.0% w/w), respectively.

Phytochemical components *of G. pictum* leaves

The results obtained from the phyto-chemical screening conducted on the ethyl acetate- and hexane extracts of *G. pictum* are presented in Table 1. Flavonoids, saponins and

Table 1 Phytochemical constituents of ethyl acetate and hexane extracts of *Graptophylum pictum*

Phytochemical type	Presence	
	Ethyl Acetate	Hexane
Alkaloids	-	+
Flavonoids	-	-
Tannins	+	+
Saponins	-	-
Steroids	+	-
Triterpenoids	-	-

(+) = present and (-) = not detected

triterpenoids were absent in both the ethyl acetate and hexane extracts of *G. pictum*. The ethyl acetate extracts contain tannins and steroids, whereas the hexane extracts contain alkaloids and tannins.

α-Glucosidase inhibitory activity of *G. pictum* leaf extracts

The α-Glucosidase inhibition activity of the ethyl acetate- and hexane extracts and acarbose is shown in Fig 1. The effective inhibition of α-glucosidase by ethyl acetate and hexane extracts of *G. pictum* were 30.68 and 49.83%, respectively. Acarbose, used as the positive control, showed a percentage inhibition value of 77.58%, under similar assay conditions.

Kinetics of α-glucosidase inhibiton

The inhibition kinetics of ethyl acetate- and hexane extracts analyzed by Lineweaver-Burk plot analysis, indicated that it is an uncompetitive and noncompetitive inhibitor with respect to PNPG for α-glucosidase, respectively (Table 2). The inhibitor concentration (%) was plotted on X-axis and 1/V $(mM/min)^{-1}$ values obtained from the Lineweaver-Burk plot was plotted on the Y axis. The ethyl acetate extract of *G. pictum* behaved as an uncompetitive inhibitor (s) for α-glucosidase (Fig 2) and resulted in reduction of Vmax from 0.174 mM/ min^{-1} to 0.042 mM/ min^{-1} with a Km change from 0.080 to 0.0005 mM. The kinetic studies showed that the hexane extract of *G. pictum* increased the maximum velocity of the enzyme activity (or Vmax) without much change in the Km values (Table 2 & Fig 3). The kinetic results of hexane extract demonstrated that mechanism of α-glucosidase inhibition was reversible, and noncompetitive in nature.

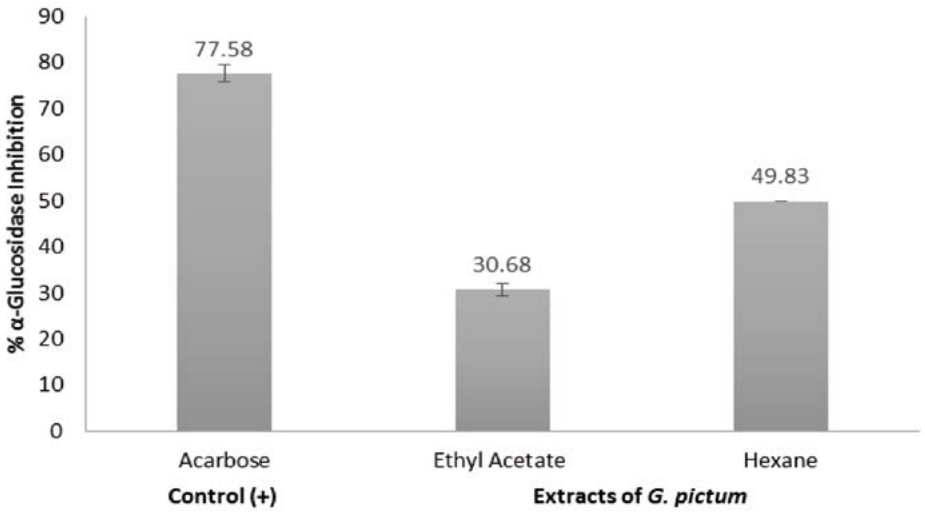

Figure 1 The inhibitory effect of ethyl acetate- and hexane extracts of *G. pictum* on α-glucosidase (positive control: acarbose (1%). The results are expressed as mean ± S.D., n=3.

Table 2 Kinetic analysis of α-glucosidase by ethyl acetate- and hexane extracts of *Graptophylum pictum*

Extract concentration (%)	Ethyl acetate extract		Hexane extract	
	Km (mM)	Vmax (mM/ min^{-1})	Km (mM)	Vmax (mM/ min^{-1})
0	0.087	0.456	0.066	0.513
2	0.080	0.174	0.078	0.327
5	0.0005	0.042	0.071	0.492

4. DISCUSSION

Diabetes mellitus is a common metabolic disorder which may eventually lead to multiple organ damage and syndromes (Rahimi *et al.* 2005). There are an assorted number of plants and plant-derived-compounds have been used in the treatment of diabetes to control blood sugar, as the synthetic antidiabetic drugs have adverse side effects in humans (Nagmoti & Juvekar 2013). Glucosidase is the key enzyme involved in hydrolysis of carbohydrate and inhibitors of this enzyme may be exploited as therapeutic approaches for controlling postprandial hyperglycemia (Shim *et al.* 2003; Bhat *et al.* 2011). Phytochemicals from plants such as phenolic compounds, saponins, flavonoids, glycosides, alkaloids *etc*. have been reported to play an important role in modulating glucosidase activity and can therefore contribute to the management of postpradial hyperglycemia (Pulok *et al*. 2006; Ani & Akhilender 2008). In the present study, experiments have been carried out to evaluate the phytochemical components and the potential of ethyl acetate- and hexane extracts of *G. pictum* in inhibiting α-glucosidase activity.

Here, we report the antidiabetic activity of ethyl acetate- and hexane extracts of *G. pictum.* The present finding of the phytochemical screening of the extracts confirmed the presence of several bioactive compounds such as alkaloids, tannins and steroids (Table 1) which could be responsible for the versatile medicinal properties of these plant extracts. This is in agreement with previous reports that phytochemicals from some plants are strong inhibitors of the α-glucosidase

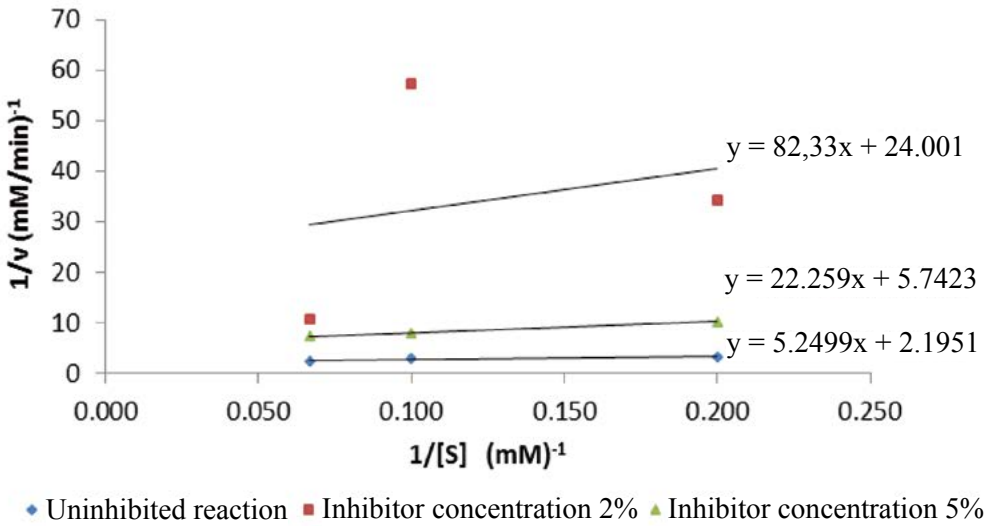

Figure 2 Lineweaver-Burk plot of α-glucosidase and PNPG with (■) 2% and (▲) 5% of ethyl acetate extract of *G. pictum*.

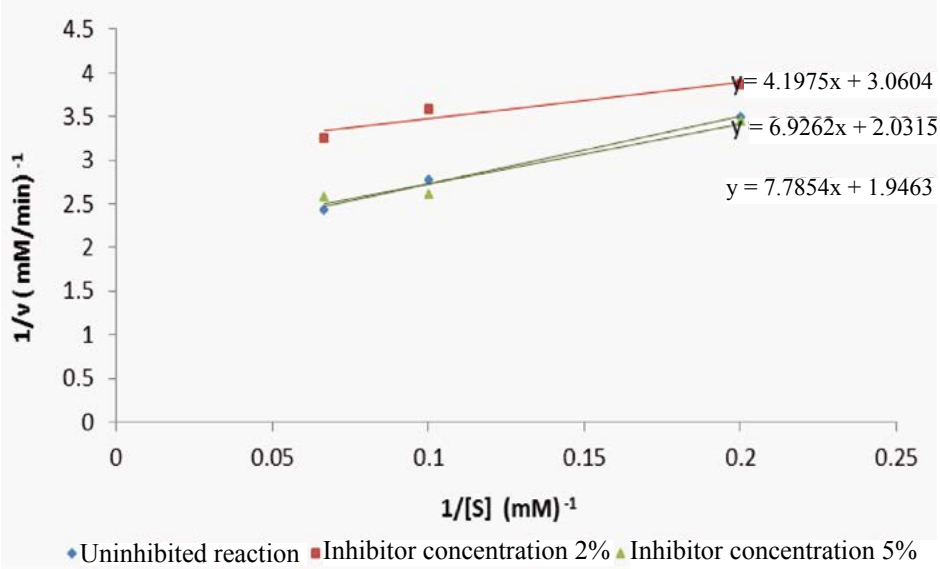

Figure 3 Line weaver-Burk plot of α-glucosidase and PNPG with (■) 2% and (▲) 5% of hexane extract of *G. pictum*.

(Kwon *et al.* 2007). To determine the mode of inhibition toward α-glucosidase, the double reciprocal plot was constructed and it indicated a non-competitive inhibition of α-glucosidase by the hexane *G. pictum* extract (Table 2, Fig 3). This suggests that the active components of the extract bind to sites other than the active site of the enzyme and combine with either free enzyme or enzyme substrate complex possibly interfering with the action of both (Mayur *et al*. 2010). The ethyl acetate extract of *G. pictum* (Table 2, Fig 2) has uncompetitive inhibitors of α-glucosidase. The commercial α-glucosidase inhibitor, acarbose, is a competitive inhibitor of the enzyme and it is required at a higher concentration to reduce the post-prandial glucose level. The uncompetitive inhibitors bind to the enzyme-substrate complex, lowering the Km and the maximum enzyme activity (Vmax) (Gurudeeban *et al*. 2012).

In conclusion, *in vitro* studies clearly indicated that ethyl acetate- and hexane extracts of *Grapthophylum pictum* had 30.68 and 49.82% inhibitory effect on α-glucosidase activity respectively. The inhibitory effect of ethyl acetate and hexane extracts of *G. pictum* on α-glucosidase may be linked to the presence of alkaloids, tannins and steroids which may be acting individually or in synergy. The chemical compounds of the plant showed significant α-glucosidase inhibitory activity. Further studies should be directed towards chemical isolation, purification and characterization, in order to elucidate the components responsible for inhibiting activity as these may have importance for the development of antidiabetic agents.

5. ACKNOWLEDGMENTS

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